

CLAIMS

1. A method of detecting a retroviral genetic recombinant having gag and pol functions comprising:

5 providing a cell suspected of having said recombinant, wherein said recombinant may be propagated in the presence of one or more helper functions to permit detection of said recombinant; and

propagating said recombinant in the presence of said one or more helper functions to thereby detect said recombinant.

10 2.. The method of claim 1 wherein said recombinant is integrated into the genome of said cell.

3. The method of claim 1 wherein said recombinant is detected using an assay.

15 4. The method of claim 3 wherein said assay is selected from one or more members of the group of assays consisting of FISH, PCR, antigen-detection, Tat transfer, Gag transfer, and mobilization.

5. The method of claim 1 wherein said recombinant comprises one or more genetic elements selected from the group consisting of retroviral cis-acting sequences and retroviral coding sequences.

20 6. The method of claim 5 wherein said retroviral coding sequence is further selected from one or more of the group of retroviral coding sequences consisting of gag, pro, pol, rt, in, gag-pro, and gag-pol.

7. The method of claim 6 wherein at least one of said retroviral sequences is a mutated sequence.

25 8. The method of claim 7 wherein said at least one mutated sequence is a retroviral coding sequence having a mutation selected from the group consisting of: silent mutations, stop codons, deletions, and insertions.

9. The method of claim 1 wherein said recombinant is capable of mobilizing a nucleic acid sequence.

10. The method of claim 9 wherein said nucleic acid sequence is selected from one or more of the group consisting of a mobilizable marker gene, a retroviral nucleic acid sequence, and said recombinant.

11. The method of claim 1 wherein said one or more helper functions comprises at least an env gene or pseudotype thereof.

12. The method of claim 10 wherein said marker gene is a selectable marker gene integrated within a chromosome of said cell.

13. The method of claim 12 wherein said marker gene encodes antibiotic resistance.

14. The method of claim 13 wherein said antibiotic is puromycin.

15. The method of claim 10 wherein said marker gene expression is controlled by a promoter, said promoter selected from the group of promoters consisting of constitutive and inducible promoters.

16. The method of claim 10 wherein said marker gene is flanked by cis-acting sequences for encapsidation, reverse transcription, and integration.

17. A method for detecting a retroviral genetic recombinant having gag and pol functions comprising:

18. providing a cell suspected of having said recombinant, said cell comprising a marker gene, said marker gene capable of being mobilized by said recombinant in the presence of one or more helper functions; and

19. supplying said one or more helper functions to mobilize said marker gene, and thereby permitting detection of said recombinant.

20. The method of claim 17 wherein said recombinant and said marker gene are each integrated into the genome of said cell, and wherein each is capable of one or more functions selected from the group consisting of expressing a gene and being mobilized.

25. The method of claim 18 wherein said at least one or more functions is detected using an assay.

20. The method of claim 19 wherein said assay is selected from one or more members of the group consisting of FISH, PCR, antigen-detection, Tat transfer, Gag transfer, and mobilization.

5 21. The method of claim 17 wherein said recombinant comprises one or more genetic elements selected from the group consisting of retroviral cis-acting sequences and retroviral coding sequences.

22. The method of claim 21 wherein said retroviral coding sequence is further selected from one or more of the group of retroviral coding sequences consisting of gag, pro, pol, rt, in, gag-pro, and gag-pol.

10 23. The method of claim 22 wherein at least one of said retroviral sequences is a mutated sequence.

24. The method of claim 23 wherein said at least one mutated sequence is a retroviral coding sequence having a mutation selected from the group consisting of: silent mutations, stop codons, deletions, and insertions.

5 25. The method of claim 17 wherein said recombinant is capable of mobilizing a nucleic acid sequence.

20 26. The method of claim 25 wherein said retroviral nucleic acid sequence is selected from one or more of the group consisting of a mobilizable marker gene, a retroviral nucleic acid sequence, and said recombinant.

27. The method of claim 21 wherein said one or more helper functions comprises at least an env gene or pseudotype thereof.

28. The method of claim 26 wherein said marker gene is a selectable marker gene integrated within a chromosome of said cell.

25 29. The method of claim 28 wherein said marker gene encodes antibiotic resistance.

30. The method of claim 29 wherein said antibiotic is puromycin.

31. The method of claim 26 wherein said marker gene expression is controlled by a promoter, said promoter selected from the group of promoters consisting of constitutive and inducible promoters.

32. The method of claim 26 wherein said marker gene is flanked by cis-acting sequences for encapsidation, reverse transcription, and integration.

33. A method for detecting a retroviral genetic recombinant comprising:
providing a cell suspected of having said retroviral genetic recombinant, said cell
comprising a marker gene, said marker gene responsive to said recombinant; and
measuring the response of said marker gene to thereby detect said recombinant.

34. The method of claim 33 wherein said marker gene is responsive to a gene product encoded by said recombinant.

35. The method of claim 34 wherein said gene product is a retroviral gene product.

36. The method of claim 35 wherein said retroviral gene product is Tat.

37. The method of claim 33 wherein said marker gene is optionally a mobilizable marker gene.

38. The method of claim 33 wherein said marker gene is an antibiotic resistance gene.

39. The method of claim 38 wherein said antibiotic resistance gene is puromycin.

40. A retroviral assay system for detecting a retroviral genetic recombinant having gag and pol functions, comprising:

a cell suspected of containing said recombinant therein, wherein propagation of said recombinant is facilitated in the presence of one or more helper functions; and means for detecting said recombinant.

41. The retroviral assay system of claim 40 wherein said means comprises one or more members from the group consisting of

- (a) expression of a marker gene in said cell, said marker gene responsive to the presence of said recombinant;
(b) mobilizing said marker gene;
(c) mobilizing said recombinant; and
(d) assaying for a product encoded or otherwise produced by said recombinant or said marker gene.

42. The retroviral assay system of claim 41 wherein said recombinant derives from a lentivirus.

43. The retroviral assay system of claim 42 wherein said lentivirus is selected from the group consisting of HIV-1, SIV, HIV-2, FIV, EIAV, and BIV, CAEV, and
5 OVINE.

44. The retroviral assay system of claim 43 wherein said lentivirus is an HIV lentivirus.

45. The retroviral assay system of claim 41 further comprising within said cell one or more helper functions needed for mobilization.

46. The retroviral assay system of claim 41 wherein said one or more helper functions includes an env element or pseudotype thereof.

47. The retroviral assay system of any of claims 41 wherein said means for detecting said retroviral genetic recombinant is the expression of a marker gene that is responsive to the presence of said retroviral genetic recombinant.

48. The retroviral assay system of claim 47 wherein said marker gene encodes a product that can be selected for under one or more environmental conditions.

49. The retroviral assay system of claim 48 wherein said marker gene is an antibiotic resistance gene.

50. The retroviral assay system of claim 49 wherein said antibiotic is puromycin.

51. The retroviral assay system of claim 41 wherein said assay is selected from the group consisting of FISH, PCR, antigen-detection, Tat transfer, Gag transfer, and gene mobilization.

52. An indicator cell for indicating the presence of a retrovirus, comprising:
25 an integrated selectable marker gene, said selectable marker gene responsive to the presence of one or more genetic elements encoded by said retrovirus; said selectable marker gene optionally a mobilizable selectable marker gene, and said retrovirus optionally a retroviral genetic recombinant.

53. The indicator cell of claim 52 wherein said one or more genetic elements
30 encodes Tat, and said selectable marker gene is driven by an LTR promoter.

54. The indicator cell of any of claim 52 wherein said indicator cell is an immortalized mammalian cell.

55. The indicator cell of claim 54 wherein said immortalized cell is selected from the group consisting of HeLa, 293T, and derivatives thereof.

56. The indicator cell of any of 52 wherein said selectable marker gene is an antibiotic resistance gene.

57. The indicator cell of claim 56 wherein said antibiotic is puromycin.

58. The indicator cell of claim 52 wherein said retrovirus is a retroviral genetic recombinant having gag and pol functions, and wherein said indicator cell optionally further comprises one or more helper functions necessary for propagating said recombinant.

59. The method, system, or indicator cell of any of the preceding claims that is used to evaluate the risk of producing a replication-competent retrovirus from a retroviral-based vector.